



UNIVERSITI PUTRA MALAYSIA

**MICROSATELLITE DEVELOPMENT AND CROSS-PHYLUM
APPLICATION FOR CHARACTERIZATION OF THE GIANT
FRESHWATER PRAWN (*Macrobrachium rosenbergii*)**

SOMAYEH HOOSHMAND

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By

SOMAYEH HOOSHMAND

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfilment of the Requirement for the Degree of Master of Science**

May 2009



*Specially dedicated to,
My beloved Father, Mother and My sister Naghmeh
For their invaluable love, understanding, tolerance, sacrifice, moral
support*

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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Chairman: Professor Tan Soon Guan

Faculty: Biotechnology and Biomolecular Science

The giant freshwater prawns, *Macrobrachium rosenbergii* locally known as udang galah is a favourite and important aquaculture candidate. Their numbers have been declining over the past decades. Hence, maintaining the genetic variability of natural populations and the identification of population structures are the main concerns of any management programme. Among the vast choice of molecular markers available to carry out a population study, microsatellites have a high degree of polymorphism and therefore have great potential for characterizing populations. The present study had three major objectives: 1) to estimate the population genetic structure in four *M. rosenbergii* populations using microsatellite markers. 2) to cross amplify the microsatellite markers developed for *Tor tambroides* on *M. rosenbergii*. 3) to isolate, develop and characterize new microsatellite markers for *M. rosenbergii* and to test them for polymorphisms.

Twenty one sets of microsatellite primer pairs were used in the initial screening. Fifteen primer pairs produced clear and reproducible amplification products in four populations of this prawn and were used to determine and compare the genetic

structures of these populations. Twenty six individuals of each population were analyzed. The number of observed alleles per locus ranged from 1-10 with an average value of 5.88 across all loci. The highest value of the mean effective allele number was 3.20 in the Linggi population and the lowest was 3.04 in the Permatang population. The highest mean observed heterozygosity was found in the Sedili population with a value of 0.67 while the Linggi population had the lowest value of 0.60. The F_{is} values indicated heterozygosity excess in three populations of Pahang, Sedili and Permatang whereas the Linggi population showed heterozygote deficiencies. The analysis of molecular variance (AMOVA), based on the 15 polymorphic common loci investigated showed that 7.79% of the variations were among populations and 92.21% of the variations were within populations. The majority of loci showed significant deviations from Hardy-Weinberg equilibrium. This might have been resulted from mutation, migration, selection and small population size. The presence of null alleles cannot be the reason for such results since their occurrence was less. The cluster analysis revealed that the Pahang and Linggi populations were the closest that was in accordance with the geographical regions from which the populations were obtained. Cross-phylum amplification studies of *Tor tambroides* were conducted on *M. rosenbergii*. The successful amplification demonstrated that microsatellite loci were conserved between these two aquatic species. This conservation of microsatellites in aquatic species may provide a valuable and cost-effective alternative to isolating microsatellite loci in every species of interest. However, in this study 10 new microsatellite loci were isolated from *M. rosenbergii* using a Random Amplified Microsatellites (RAMs) based technique which was an efficient and reliable method. These newly developed microsatellite loci were tested for polymorphisms.

In conclusion these four wild populations of *M. rosenbergii* consist of Pahang, Linggi, Sedili and Permatang showed high variability of heterozygosity assessed by 15 polymorphic microsatellites. Therefore, the results were informative for the researchers to make sound decisions in managing the wild populations for their future use in broodstock genetic improvement programs and also for the use in constructions. All ten microsatellite loci that were developed should be tested on larger sample sizes of each population and on more populations.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

**PEMBANGUNAN MIKROSATELIT DAN AMPLIFIKASI SILANG-FILUM
UNTUK PENCIRIAN UDANG AIR TAWAR GERGASI (*Macrobrachium
rosenbergii*)**

Oleh

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May 2009

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Udang air tawar, *Macrobrachium rosenbergii* atau nama tempatannya udang galah adalah sangat digemari dan merupakan calon akuakultur yang penting. Bilangannya telah berkurangan sejak sedekad lalu. Justeru, kelestarian kepelbagaian genetik populasi semulajadi dan mengenalpasti struktur populasi adalah menjadi keutamaan bagi program pengurusan.

Di kalangan pelbagai pilihan penanda molekul yang ada untuk kajian populasi, mikrosatelit mempunyai darjah polimorfisme yang tinggi dan oleh itu ia berpotensi untuk pencirian populasi.

Dalam kajian ini terdapat tiga objektif utama 1) untuk mengangar struktur genetik populasi pada empat populasi *M. Rosenbergii* dengan menggunakan penanda mikrosatelit. 2) untuk menjalankan amplifikasi silang penanda mikrosatelit yang dibangunkan untuk *Tor tambroides* pada *M. Rosenbergii*. 3) untuk memencil,

membangun dan mencirikan penanda mikrosatelit yang baru untuk *M. Rosenbergii* dan untuk menguji kepolimorfisme mereka.

Dua puluh satu set pasangan penanda mikrosatelit telah digunakan pada pengimbasan permulaan. Lima belas pasangan telah menghasilkan produk amplifikasi yang terang dan boleh terhasil semula pada empat populasi udang ini dah telah digunakan untuk menentukan dan membandingkan struktur genetik populasi ini. Dua puluh enam individu dari setiap populasi telah dianalisis. Jumlah alel tercerap setiap lokus berjulat antara 1-10 dengan purata 5.88 merentasi kesemua lokus. Nilai paling tinggi untuk purata keberkesanan alel adalah 3.20 di populasi Linggi dan yang paling rendah bernilai 3.04 pada populasi Permatang. Nilai tertinggi untuk purata heterozigositi tercerap telah dijumpai di dalam populasi Sedili dengan nilai 0.67 manakala populasi Linggi mempunyai nilai yang paling rendah iaitu 0.60. Nilai Fis menunjukkan lebih heterozigositi di tiga populasi iaitu Sungai Pahang, Sedili dan Permatang manakala Populasi Linggi menunjukkan kekurangan heterozigot. Analisis varians molekul (AMOVA) berdasarkan 15 lokus polimorfik biasa yang dikaji menunjukkan bahawa 7.79% daripada variasi adalah di kalangan populasi dan 92.21% variasi adalah di dalam populasi. Majoriti lokus menunjukkan lencongan yang signifikan daripada persamaan Hardy-Weinberg. Ini mungkin disebabkan oleh mutasi, migrasi, pemilihan dan saiz populasi yang kecil. Kehadiran alel null tidak boleh dijadikan penyebab kerana kewujudannya yang kurang. Analisis kelompok menunjukkan bahawa populasi Pahang dan Linggi adalah yang paling dekat dan ini bersesuaian dengan kawasan geografi daripada mana kedua-dua populasi ini diperolehi.

Kajian amplifikasi silang-filum (Cross-phylum) *Tor tambroides* telah dijalankan ke atas *M. rosenbergii*. Kejayaan amplifikasi ini menunjukkan bahawa lokus mikrosatelit adalah terpelihara di antara kedua-dua spesies akuatik ini. Pemuliharaan mikrosatelit ini dalam spesies akuatik menyediakan alternatif yang berharga dan menjimatkan kos untuk memencilkan lokus mikrosatelit dalam setiap spesies yang ingin dikaji.

Walaupun, dalam kajian ini 10 lokus mikrosatelit baru telah dipencilkan daripada *M. rosenbergii* dengan menggunakan kaedah berasaskan Amplifikasi Mikrosatelit Rawak (Random Amplification Microsatellite, RAMs), merupakan kaedah yang efisien dan bagus. Loci mikrosatelit yang baru dibina ini telah diuji kepolimorfismunya.

Kesimpulannya, empat populasi liar *M. Rosenbergii* ini terdiri dari Pahang, Linggi, Sedili dan Permatang telah menunjukkan kepelbagaian heterozigositi yang tinggi dijana dari 15 loci mikrosatelit polimorfik. Oleh itu, keputusan ini amat berinformasi untuk para penyelidik untuk membuat keputusan didalam menguruskan populasi liar untuk kegunaan program pembaikan baka ternakan di masa hadapan dan juga untuk kegunaan pembangunan. Kesemua sepuluh loci mikrosatelit yang telah dibina harus di uji terhadap sampel size yang lebih besar untuk setiap populasi dan populasi yang lebih banyak.

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I certify that a Thesis Examination Committee has met on 15th May 2009 to conduct the final examination of Somayeh Hooshmand on her thesis entitled “Microsatellite Development and Cross-phylum Amplification for Characterization of the Giant Freshwater Prawn (*Macrobrachium rosenbergii*) in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U. (A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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DECLARATION

I hereby that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

SOMAYEH HOOSHMAND

Date: 19/6/2009

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LIST OF ABBREVIATIONS

µg	microgram
µl	microlitre
pmol	picmole
10X	ten times
1X	one time
A	adenosine
bp	base pair
C	cytosine
dATP	deoxyadenosine triphosphate
dCTP	deoxycytidine triphosphate
ddH ₂ O	double distilled water
dGTP	deoxyguanosine triphosphate
DNA	deoxyribonucleic acid
dNTPs	deoxyribonucleotide triphosphate
dTTP	deoxythymidine triphosphate
EDTA	ethylenediamine tetraacetic acid
g	gram
G	guanosine
h	hour
kb	kilobase
kg	kilogram
M	molar
mg	milligram

Mg/ml	milligram per millilitre
MgCl ₂	magnesium chloride
min	minute
mL	millilitre
mM	millimolar
mm	millimetre
ng	nanogram
nm	nanometre
°C	degree Celsius
OD	optical density
PCR	polymerase chain reaction
RNA	ribonucleotide acid
s	second
TBE	tris-borate-EDTA buffer
U	unit
UV	ultraviolet
V	volt

CHAPTER 1

INTRODUCTION

Due to the increasing human population and their high demand for food security and protein shortage in their daily lives, attention is now being focused more on aquaculture as a source of food. Prawns are most valuable and probably the most popular seafood with high protein content. Several factors have made it an important aquaculture candidate such as: short farming period, easy to handle, high disease resistance and very tasty.

The giant freshwater prawn, known as *Macrobrachium rosenbergii* (De Man, 1879), is the largest known palaemonid in the world. The modern aquaculture of this species began in the early 1960s through the work of Shao-Wen Ling, a Food and Agriculture Organization (FAO) expert, when he and his team discovered that the larvae of *M. rosenbergii* needed brackish water for survival (Ling and Mumaw, 1977).

Today, they are one of the most commercially important crustaceans and are widely cultured all over the world. In the last decade, the average *M. rosenbergii* production rose by some 9-35.48% in quantity and 19.68-24.5% in value. In 1993, the overall production was 17,164 tonnes, worth US\$ 116,799,000 and in 2005 it reached 205,033 tonnes with a net value of US\$ 896,263,000 (FAO, 2007). Giant freshwater prawn farming is thus a major contributor to global aquaculture, both in terms of quantity and value. The giant freshwater prawn *M. rosenbergii* which is found in Asia but has also

been introduced into South and North America is a favorite protein source for Malaysians of various cultural and racial backgrounds. Thus, it has become the most valuable prawn species for aquacultural development in Malaysia, with its natural distribution in almost all the major river systems in the country.

Malaysia has drawn up an aquaculture development plan for the period 1996-2010 to outline strategies to develop commercial aquaculture in a sustainable manner (NACA, 1996). Hence the biological and economic importance of *M. rosenbergii* prompted researchers to pay attention to the population genetic structure of this species. On the other hand, this species has faced to the declining numbers of their populations and also loss of genetic diversity. Recently, in order to cultivate commercial cultures, the wild stocks have been used in many areas in Malaysia. Therefore, repeated harvesting from the wild stocks might be eventually led to extinction and loss of genetic diversity.

Understanding the population genetic structure and maintaining the genetic variability in declining populations are the primary concerns of conservation biology in aquaculture and fisheries management. This is because higher genetic variation plays a huge role to enhance the probability of population survival and improve the fitness of individuals (Zoller *et al.*, 1999). Genetic variation is an important factor in the process of evolution in a natural population. It is an important component of biodiversity and should be conserved for its intrinsic value (Ferguson *et al.*, 1995).

Natural populations act as gene banks in nature. Once the natural genetic variation is lost, it can lead to overall extinction of the population or species. Genetic variation can